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Transcriptional regulation by changes in tonicity

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Transcriptional regulation by changes in tonicity. Most organisms respond to a hypertonic environment by accumulating small organic solutes. In contrast to high concentrations of electrolytes, the small organic solutes do not perturb the activity of enzymes and other macromolecules within the cell. When the renal medulla becomes hypertonic during antidiuresis, multiple signaling pathways are activated. Here, we review the role of tonicity responsive enhancers (TonE) binding protein (TonEBP), a transcription factor activated in hypertonic cells. The activation of TonEBP by hypertonicity results from its translocation to the nucleus as well as an increase in TonEBP mRNA and protein. TonEBP may have a role beyond the response to tonicity since it is highly expressed in activated lymphocytes and in developing tissues.

The concentration of electrolytes within renal medullary cells remains remarkably constant despite large changes in the effective osmotic activity (tonicity) of the interstitium of the renal medulla (sodium varies between 140 mmol/L in hydrated rats and more than 400 mmol/L in antidiuretic rats) [1]. The stability of the intracellular electrolyte concentration results from the tonicity-regulated accumulation of specific small organic solutes that, in contrast to changing concentrations of electrolytes, do not perturb the activity of proteins and other macromolecules within cells [2]. The nonperturbing organic solutes that are accumulated by cells in the hypertonic medulla and in a variety of cultured cells are polyhydric alcohols (myoinositol and sorbitol), methylamines (betaine and glycerophosphorylcholine), and amino acids (taurine, alanine, proline, and to a lesser extent, other amino acids). Nonperturbing solutes are accumulated as the result of changes in the activity of rate-limiting, sodium-coupled transporters that carry the organic solutes into cells against steep concentration gradients (myoinositol, betaine, amino acids) and changes in activity of rate-limiting enzymes that catalyze solute synthesis (sorbitol) or degradation (glycerophosphorylcholine). Molecular cloning of the cDNAs for the rate-limiting proteins revealed that the increases in activities of those proteins result from a hypertonicity-induced increase in the transcription of their genes and the resulting increase in synthesis of the proteins. The single exception is a transcription-independent decline in the activity of glycerophosphorylcholine phosphodiesterase, which degrades glycerophosphorylcholine [3]. Within the past few years, we have gained further insight into the molecular mechanism of the regulation of transcription by hypertonicity.

The regulation of transcription of most nonhousekeeping genes depends on specific DNA elements that are typically located in the region 5′ to their promoter. Tonicity responsive enhancers (TonEs) have been identified in that region of the gene for the betaine transporter (BGT1) [4] and subsequently in that region of the gene for aldose reductase (AR), where they were designated osmotic response elements (ORE) [5–7], and in distant 5′ regions of the SMIT gene [8]. The TonE consensus sequence TGGAAANNYNY confers transcriptional stimulation by hypertonicity on reporter genes with TonEs in their 5′ region. In addition, the nucleus of hypertonic cells contains a protein that binds to radiolabeled TonEs in vitro in electrophoretic mobility shift assays (EMSAs) with far more activity than that found in the nucleus of isotonic cells. In hypertonic cells, specific G residues within the TonEs of BGT1 and SMIT are protected from methylation in so-called in vivo footprinting assays, whereas in isotonic cells, those G residues are methylated [8]. The changes in EMSA mobility and the protection from methylation in hypertonic cells suggest that the activation of transcription conferred by TonEs is the result of the binding of one or more proteins to TonEs.

Using TonEs as bait in yeast one-hybrid assays, we cloned the cDNA for a novel transcription factor, TonE binding protein (TonEBP), a large protein of approximately 160 kD [9]. Unexpectedly, mRNA for TonEBP is expressed in every tissue examined. The highest levels of expression are found in the renal medulla, brain, and heart. Figure 1 presents cartoon diagrams of TonEBP and the NFAT transcription factors, which play a key role in the regulation of transcription in activated lymphocytes, heart, and mesenchymal cells. The TonEBP sequence carboxy to the DNA binding domain contains many glutamine residues, a feature commonly found in

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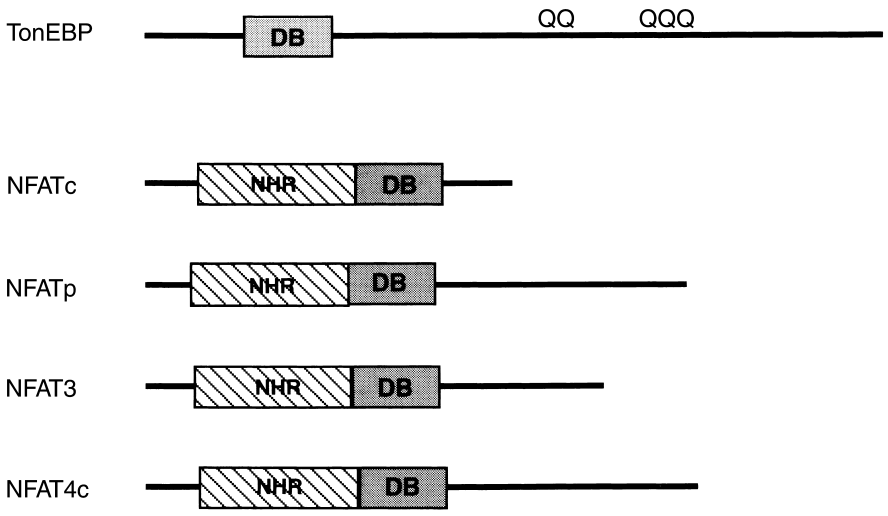

Fig. 1. Schematic comparison of the primary sequences of tonicity responsive enhancer binding protein (TonEBP) and the NFAT family. The grey boxes labeled "DB" denote DNA binding domains, while the striped boxes labeled "NHR" denote the NFAT homology region. NHR binds calcineurin and is dephosphorylated when calcineurin is activated. Note that NHR is not found in TonEBP. "QQ" and "QQQ" denote stretches of glutamine's transcriptional regulation by changes in tonicity.

the activator region of transcription factors. The hypothesis that the glutamine-rich region mediates transcriptional activation was supported when we expressed a peptide lacking the presumed activator domain. The truncated protein behaved as a dominant negative; that is, it inhibited the transcriptional activation of reporter genes in hypertonic cells [9]. The DNA binding domain of TonEBP has a 45% identity to the DNA binding domains of the NFAT family of transcription factors, which share about 80% identity in that region. The greater identity within the DNA binding domain of NFATs includes a region that interacts with the transcription factor AP1. TonEBP lacks the region for interaction with AP1 and, in contrast to the NFAT transcription factors, stimulates transcription in the absence of activated AP1 [10]. Using a variety of strategies, other laboratories have subsequently cloned TonEBP and called it NFAT5 [10], NFATL1 [11], NFATz [12], and OREBP [13]. In contrast to the members of the NFAT family, TonEBP has no sequence homology to NFAT proteins outside of the DNA binding domain. Specifically, TonEBP lacks a sequence that is located amino terminal to the DNA binding domain, is phosphorylated by a nuclear protein kinase and directs the export of NFATs to the cytoplasm. In quiescent cells, NFATs are cytosolic. When lymphocytes are activated and cell calcium rises, NFATs are dephosphorylated by the calcium-calmodulin–sensitive phosphatase calcineurin and move to the nucleus where they regulate expression of specific genes. Activation of NFATs is inhibited by cyclosporine A and by FK506, which inhibit the phosphatase activity of calcineurin. Although there is a preliminary report of inhibition of the transcriptional response to hypertonicity by cyclosporine A and by FK506 using high concentrations of these agents (abstract; Sheikh-Hamad et al, *J Am Soc Nephrol* 11:47A, 2000), we found that cyclosporine A and FK506 had no effect on the stimulation by hypertonicity of BGT1 activity and had divergent effects on the stimulation of SMIT activity [14]. Another laboratory found that concentrations of cyclosporine A or FK506 that clearly inhibited calcineurin activity in lymphocytes did not inhibit the transcriptional response to hypertonicity in the same cells [11].

TonEBP undergoes a number of changes when it is activated by hypertonicity. First, TonEBP is phosphorylated when cells are shifted to a hypertonic medium [15]. Most of the phosphorylation occurs at serine residues, but also there is increased tyrosine phosphorylation. Since TonEBP contains 197 serine residues and 13 tyrosine residues, it will be difficult to identify the important phospho-residues. Using immunofluorescence and immunoprecipitation from cell fractions, we found that in isotonic cells, approximately half of the cell's TonEBP is in the cytoplasm and half in the nucleus. Within an hour of exposure to hypertonicity, cytoplasmic TonEBP diminishes, and nuclear TonEBP increases. After 8 to 12 hours, almost all of the TonEBP is nuclear [9]. In view of the similar time course for phosphorylation of TonEBP and its movement to the nucleus, we hypothesize that the phosphorylation plays a role in moving TonEBP to the nucleus, but this remains to be tested. In vitro binding assays showed that the association of TonEBP with TonE enhancer sequences is not affected by phosphorylation. Proteasome activity is also important in the regulation of TonEBP by hypertonicity. When proteasome protease activity is inhibited, movement of TonEBP to the nucleus is decreased, as is the increase in nuclear TonEBP activity [16]. In analogy to the regulation of nuclear factor-κB (NF-κB), there may be a cytosolic protein that limits TonEBP movement to the nucleus in control cells that is degraded by proteasomes in hypertonic cells, but for the moment this is only specula-

tion. In addition to translocation to the nucleus, activation by hypertonicity also evokes an increase in total cell TonEBP. The increase in TonEBP protein follows an increase in TonEBP mRNA abundance, which is probably the result of stimulating the transcription of TonEBP. Unfortunately, the very low level of transcription of TonEBP precludes assessment of transcription by classic nuclear run-off assays. The regulation of TonEBP by tonicity is bidirectional. When cells are switched from a hypertonic to an isotonic medium, the degradation of TonEBP mRNA and protein is not affected by tonicity, as they decay with half-lives of approximately 6 and 10 hours, respectively. Since TonEBP has a detectable level of activity in isotonic cells, we examined its status in cells adapted to growth in medium made hypotonic by removing most of the NaCl in the medium. The level of TonEBP fell in hypotonic cells, and nuclear immunostaining decreased far more than in cytosolic cells [17]. We return to these observations later when speculating about the regulation and function of TonEBP.

There is a large gap in understanding how hypertonicity regulates TonEBP. First, how is hypertonicity detected? The strategy of maintaining intracellular ionic activity nearly constant by accumulating or losing organic osmolytes as external tonicity changes is an old one. Like the animal cells we study, bacteria, yeast, and most plant and animal cells accumulate amino acids and polyhydric alcohols when exposed to a hypertonic environment [2]. Some bacterial osmolyte transporters are directly stimulated by hypertonicity [18, 19]. The initial sensor in yeast is a two-component histidine kinase located in the plasma membrane [20]. The sensor initiates a multistep phosphorelay cascade that activates a p38 mitogen-activated protein (MAP) kinase homologue, HOG kinase, which regulates the rate of transcription of a number of genes. Similar sensors have not been identified in animal cells, although there is evidence that animal cells detect changes in cell size or shape within minutes after exposure to a hypertonic environment [21, 22]. Since most cells return to near their original isotonic size within 30 minutes as a result of a regulatory volume increase mediated by ion uptake, it is unlikely that the activation of TonEBP, which occurs over hours, is mediated by cell size or shape. Multiple changes, including the rapid induction and suppression of many genes, occur when cells are abruptly shifted to a hypertonic environment. The cell cycle is arrested, and many regulatory pathways are activated or inhibited [23, 24]. Indirect evidence suggests that the sensor that regulates activation of TonEBP may detect intracellular ion activity. The activation of transcription by hypertonicity is reduced as cells accumulate organic osmolytes and cell ion concentrations return to those of isotonic cells. In cells in hypertonic medium, there is a close correlation between the increase in total cell cation concentration and the increase in activity of AR [25]. Also, inhibition or stimulation of osmolyte accumulation by the removal or addition of transporter substrate prolongs or hastens the decline in the abundance of hypertonicity-induced taurine transporter [26] or AR mRNA [27].

There are many unanswered questions regarding TonEBP. TonEBP mRNA is expressed at low levels in most cells in culture and in virtually all tissues that have been sampled. However, only the kidney medulla normally experiences marked changes in tonicity. Does TonEBP regulate processes other than those involved in the response to hypertonicity? The low level of expression of TonEBP in isotonic cells is reduced further when cells are adapted to growth in hypotonic medium, as though a hypotonic state is the null condition. Perhaps the levels of TonEBP expression in isotonic cells are based on life in more hypotonic environments experienced by ancestral precursor cells. A recent report that TonEBP, like some NFAT transcription factors, is induced in lymphocytes by ConA or by ionomycin plus a phorbol ester is intriguing [11]. In addition, TonEBP is expressed at much higher levels in embryonic tissues than in adult tissues [11]. Does TonEBP play a role in development?

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